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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

Through a collaborative research program involving Utah State University and the Cancer Research Center of Hawaii, we have discovered that the sponge-derived macrolides laulimalide and isolaulimalide are potent cytotoxins with paclitaxel-like antimicrotubule-stabilizing activity. Laulimalide is a potent inhibitor of cellular proliferation with an IC_{50} in the low nanomolar range and it maintains activity against a drug resistant, P-glycoprotein over-expressing ovarian cancer cell line. Laulimalide represents a lead compound for new class of microtubule-stabilizing agents with activities that may prove therapeutically useful for the treatment of breast cancer. The aim of this project is to utilize a combinatorial solid-phase synthetic approach for the construction of a library of laulimalide analogs for structure activity relationship (SAR) studies.

In an effort to discover a new chemotherapeutic agent for the treatment of breast cancer we propose to do the following: 1) transfer our current solution-phase synthetic approach to solid phase, 2) using a split and pool strategy, prepare 260 laulimalide analogs, 3) test laulimalide analogs for microtubule-stabilizing activity, cytotoxicity against both drug-sensitive and drug-resistant breast cell lines, and 4) submit active analogs to the NCI for screening in the 60-cell line.

Work to-date has been concentrated on: 1) the improvement of our solution phase laulimalide synthetic methods for efficient conversion to the solid phase, 2) the synthesis of structural analogs of fragments B and C, and 3) the solid-phase synthesis of laulimalide that will then be used for the combinatorial synthesis of analogs

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breast cancer, laulimalide, microtubule-stabilizing agents

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Introduction

In 1999 we reported that the marine macrolides laulimalide (**1**) is a new Taxol-like microtubule-stabilizing agents with activity against drug resistant cancer cells,¹ It therefore represents a new class of microtubule-stabilizing agent with activities that may prove therapeutically useful. The purpose of the proposed study is to produce an effective new agent for the treatment of breast cancer. With the goal of discovering a new chemotherapeutic agent for the treatment of breast cancer, we proposed to do the following: (a) transfer our current solution-phase synthetic technology to the solid phase; (b) using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity; (c) screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines; and (d) submit active analogs to the NCI for screening in the 60-cell line assay. Our future goals include the selection of the most promising analogs for scale-up synthesis and *in vivo* testing.

Body

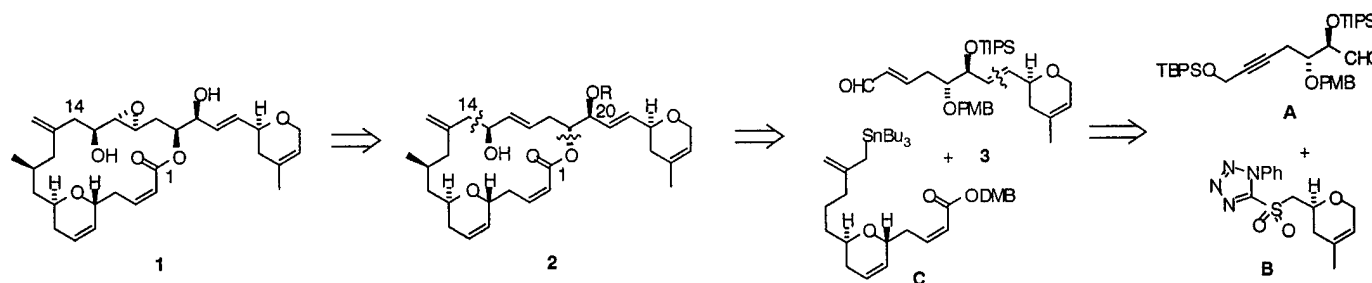
Research accomplishments for year 2 are described below for each of the Tasks in the approved Statement of Work.

Task 1. To adapt solution-phase synthetic technology to use on the solid phase (months 1-12)

- Establish conditions for linking compounds to resin and for cleaving products from resin
- Optimize reaction times/conditions for solid phase, starting with conditions established for solution phase
- Synthesize laulimalide on solid phase

During year 2, we have completed much of Task 1. We have: 1) refined our synthetic route to fragment **A.1**, as described in our year 1 annual report,² 2) improved the reaction for coupling fragments **3** and **C**, 3) developed methods for linking compounds to the resin and for cleavage from the resin, and 4) we are making good progress toward the solid-phase synthesis of laulimalide. Our current retrosynthesis is shown in Scheme 1. Salient features include: 1) moving the epoxidation reaction to the end of the synthesis, which allows epoxy- and desoxy-analogs to be diverged late in the synthesis, 2) application of an asymmetric allylation reaction using Keck's BINOL/Ti(*i*OPr)₄ conditions for coupling compound **3** and fragment **C**, and the development of a Julia-Kocienski olefination reaction for the coupling of **A** and **B**.² Work is in progress optimizing a Horner-Wadsworth-Emmons reaction for the coupling of **A** with **B** analogs.

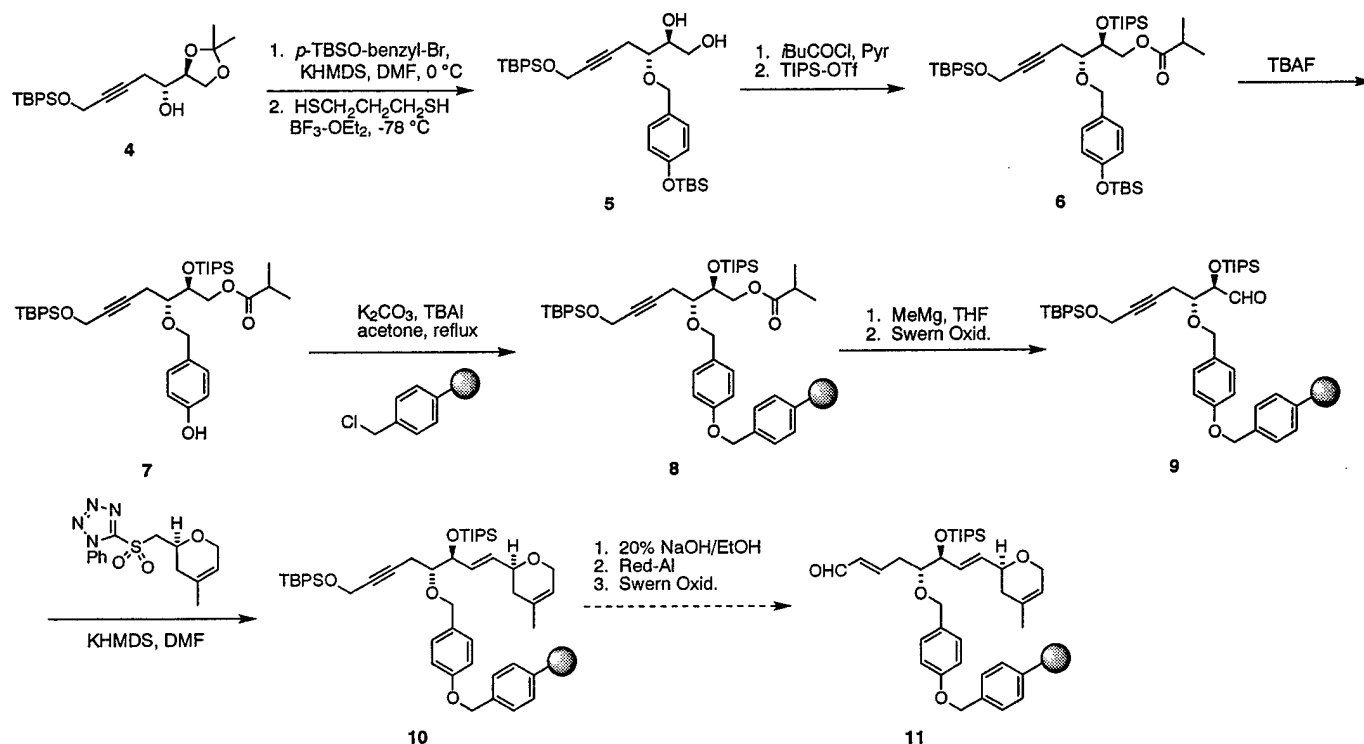
Scheme 1. Revised Retrosynthesis of Laulimalide.



We have successfully prepared compound **7** and attached it to Merrifield resin (see Scheme 2). The quantity of compound attached was estimated both by the mass of compound recovered upon cleavage with

DDQ and the quantity of **7** remaining after the attachment reaction. The isobutyrate ester in resin-bound **8** was then removed using a Grignard reaction and the resulting alcohol was oxidized to give **9**, which was ready for side chain attachment. Our solution-phase synthesis utilizes a pivaloate ester instead of the isobutyrate; however, we found the pivaloate difficult to remove while on the solid-phase. Side chain attachment was accomplished in yield and *E/Z* ratio similar to that obtained in solution, when a large excess of sulfone (**7** eq.) was used. The last three steps are currently being attempted.

Scheme 2. Solid Phase Synthesis.

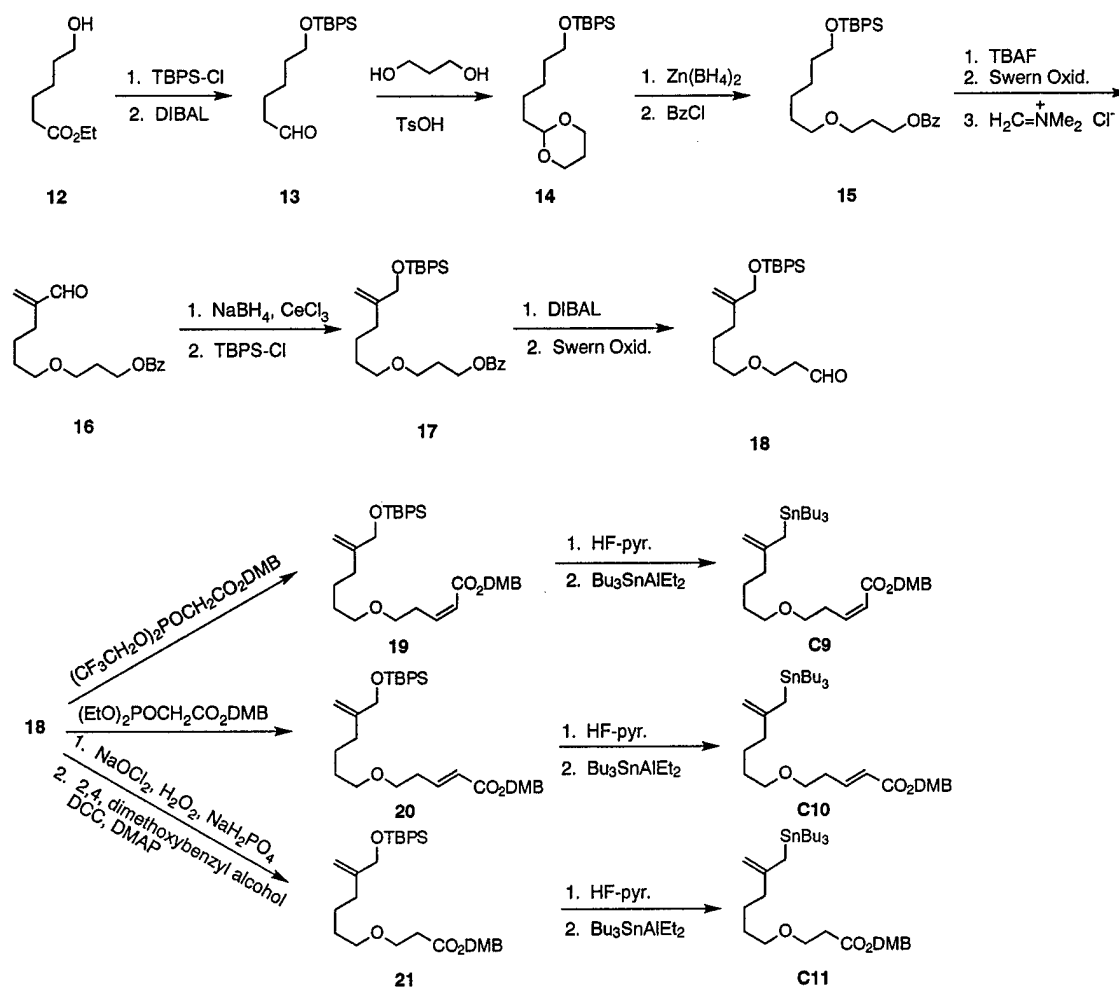


Task 2. Using a split-and-pool strategy, prepare a library of up to 260 laulimalide analogs that are designed for 1) improved stability, 2) ease of synthesis, and 3) structural diversity.

- Synthesize subunits **A2**, **B1-B10**, **C1-C15**. (months 1-12)
- Adapt solid phase reactions for use in micro-reactors (months 6-12)
- Phase I combinatorial synthesis of 60 laulimalide analogs (months 13-18)
- Phase II combinatorial synthesis of 200 laulimalide analogs (months 24-36)

Subunit synthesis has continued during year 2. In addition to the **B** subunits and **C3** that were reported for year 1, we have synthesized subunits **C9 – C14** (Schemes 3 and 4) and we are currently preparing **C6 – C8** using analogous methodology.

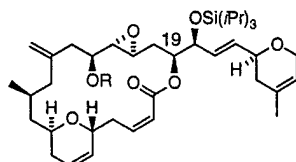
The solid phase synthetic results presented under Task 1 have been accomplished using IRORI micro-reactors. Combinatorial syntheses will be undertaken upon completion of the solid phase synthesis of laulimalide.

Scheme 3. Preparation of C9 – C11 Subunits.

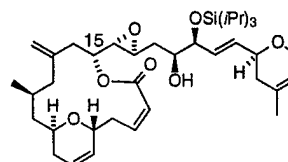
Task 3. Screen laulimalide analogs for tubulin polymerization promoting activity and cytotoxicity toward drug-sensitive and drug-resistant breast cancer cell lines.

- Establish benchmark activities for laulimalide in assays (months 13-18)
- Test Phase I combinatorial library (months 19-24)
- Test Phase II combinatorial library (months 25-36)

Two laulimalide analogs have been submitted for biological testing. The first (**32**) has both hydroxyl groups capped and the second (**33**) includes a smaller macrocyclic ring, resulting from macrolactonization with the C15 alcohol rather than the C19 alcohol. Results are pending.



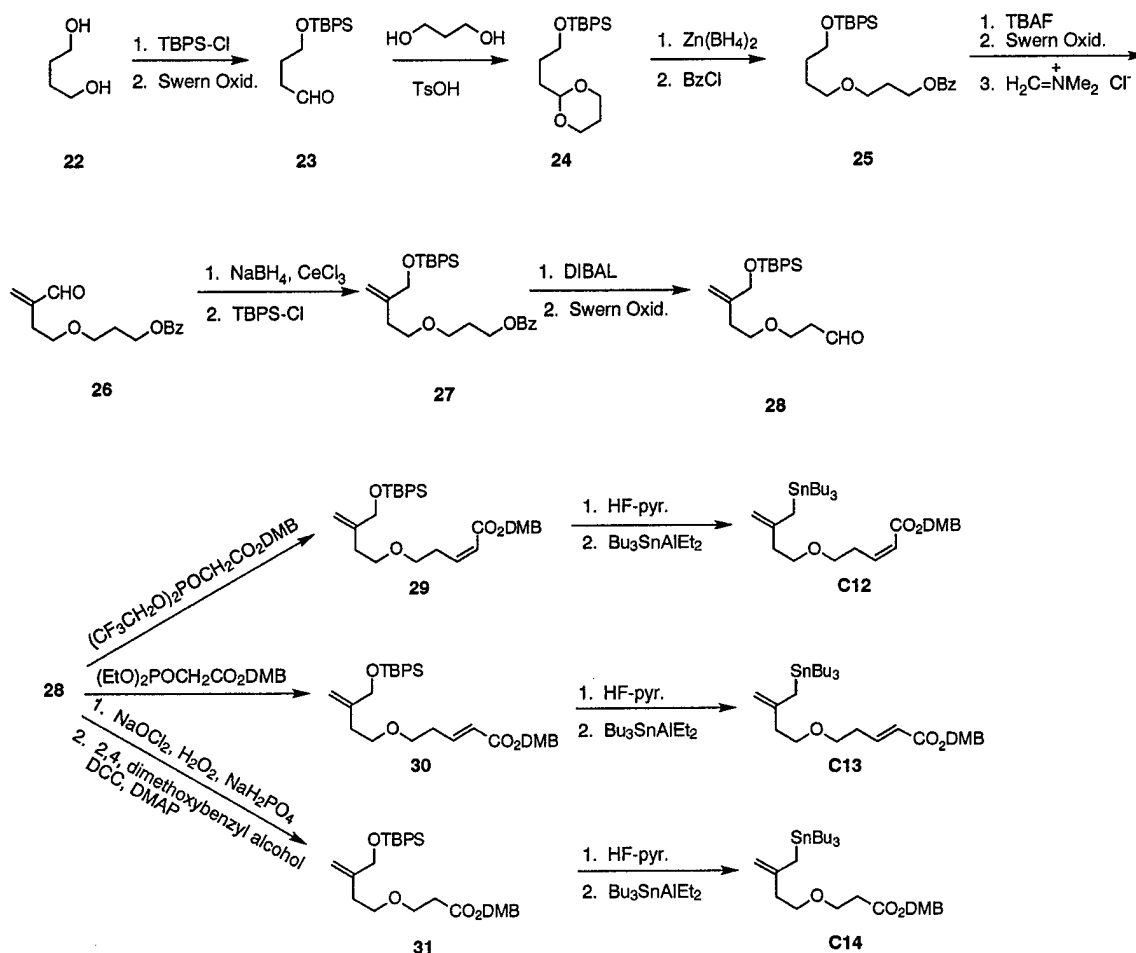
32 R = *p*-nitrobenzoate



33

Task 4. Submit active analogs to the NCI for screening in the 60-cell line assay (months 19-36)

No progress yet.

Scheme 4. Synthesis of substructure C3.**Key Research Accomplishments**

- Improved synthesis of compound 3.
- Successful adaptation of solution-phase methods to the solid-phase synthesis of intermediate 11.
- Preparation of subunits C9 – C14.

Reportable Outcomes*Publication*

Sivaramakrishnan, A.; Nadolski, G.T.; McAlexander, I.A.; Davidson, B.S. An Improved Synthesis of the C15-C28 Fragment of Laulimalide. *Tetrahedron Lett.* **2002**, *43*, 213-216.

Meeting Presentation

Bradley S. Davidson, Geoffry T. Nadolski, B. Travis Messenger, A. Sivaramakrishnan, Ian McAlexander, and Brian Hathaway, "Synthetic Approaches to the Microtubule-Stabilizing Agent Laulimalide," 10th International Symposium on Marine Natural Products, Nago, Okinawa, June 2001.

Bradley S. Davidson, Geoffry T. Nadolski, and Eric Tanifum, "Solid-Phase Synthetic Approaches for the Preparation of Laulimalide Analogs," International Meeting of the American Society of Pharmacognosy, New Brunswick, New Jersey, July 2002.

Dissertation

Geoffry T. Nadolski, "Solution And Solid-Phase Approaches for the Synthesis of Laulimalide, A Sponge-Derived Microtubule Poison," Utah State University, 7/2002. Job placement: Hawaii Biotech, Aiea, Hawaii.

Conclusions

During year 2, work has progressed on developing the solid-phase methodology necessary for the combinatorial synthesis of libraries of analogs. In addition, the fragment analogs needed for the combinatorial synthesis are being prepared.

References

1. Mooberry, S.L.; Tien, G.; Plubrukarn, A.; Davidson, B.S. Laulimalide and Isolaulimalide, New Paclitaxel-Like Microtubule-Stabilizing Agents. *Cancer Res.* **1999**, *59*, 653.
2. Sivaramakrishnan, A.; Nadolski, G.T.; McAlexander, I.A.; Davidson, B.S. An Improved Synthesis of the C15-C28 Fragment of Laulimalide. *Tetrahedron Lett.* **2002**, *43*, 213-216.



An improved synthesis of the C15–C28 fragment of laulimalide

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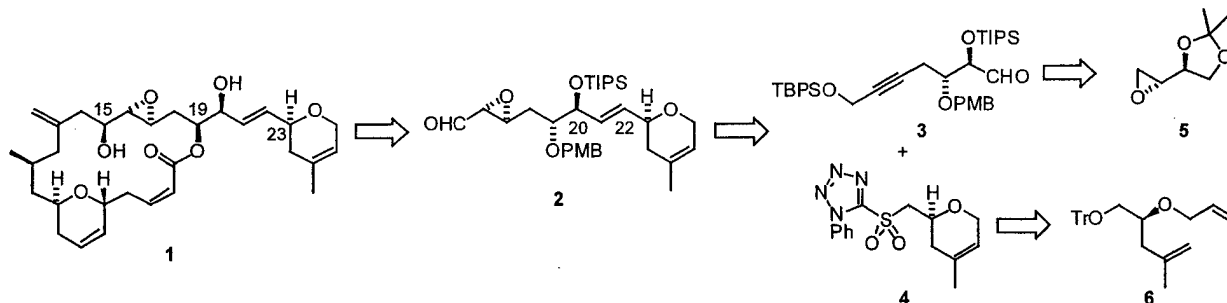
Abstract—The C₁₅–C₂₈ fragment of the paclitaxel-like antimicrotubule agent laulimalide has been synthesized in 12 linear steps from known epoxide **5**, with an overall yield of 16%. The methyldihydropyran ring of the side chain was efficiently prepared using ring-closing olefin metathesis chemistry. The 19,20-diol stereochemistry originates in starting material **7** and the side chain was appended using a Kocienski-modified Julia coupling. © 2002 Elsevier Science Ltd. All rights reserved.

As part of a program aimed at the discovery of new antimicrotubule agents, we recently identified the marine macrolide laulimalide (**1**)¹ as a new paclitaxel (TaxolTM)-like microtubule-stabilizing agent.² Like paclitaxel, laulimalide induces the dose-dependent reorganization of cellular microtubules, as well as the formation of abnormal mitotic spindles. It stimulates the polymerization of tubulin in the absence of polymerization promoters such as glycerol and GTP. Laulimalide is a potent inhibitor of cellular proliferation with IC₅₀ values in the low nanomolar range against drug sensitive cell lines and, in contrast to paclitaxel, it retains activity against SKVLB-1 cells, a P-glycoprotein over-expressing multidrug resistant ovarian cancer cell line, suggesting that it is a poor substrate for transport by P-glycoprotein. Furthermore, laulimalide triggers apoptotic cell death. Laulimalide, therefore, represents a new class of microtubule-stabilizing agent, with activities that may prove therapeutically useful, placing it within an exclusive group of compounds that, in addition to the taxanes, includes only the marine metabolites

discodermalide³ and eleutherobin⁴ and the microbial metabolites the epothilones.⁵

Laulimalide has attracted significant recent attention from synthetic organic chemists, with five groups having published a total of 16 papers describing their synthetic efforts related to laulimalide.⁶ The first total synthesis of laulimalide was reported,^{6h} along with a follow-up paper describing an improved macrocyclization approach,⁶ⁱ by the Ghosh group. Additional completed syntheses have now been published by the Mulzer^{6o} and Paterson groups.^{6p} In this communication, we would like to describe our second generation synthesis of the C15–C28 portion of laulimalide.

Our revised retrosynthetic analysis is shown in Scheme 1. It involves dividing the C15–C28 fragment (**2**) into two pieces, aldehyde **3** (C15–C21) and sulfone **4** (C22–C28), which we proposed to connect via a Julia/Kocienski coupling reaction.⁷ Fragment **3** was to be prepared in a straightforward manner from known epoxide **5**,⁸



Scheme 1. Retrosynthetic analysis.

* Corresponding author.

available in seven steps from L-ascorbic acid.⁹ Sulfone **4** is available from diene **6**, an intermediate in our previous synthesis,^{6k} using ring-closing olefin metathesis chemistry.¹⁰ This approach differs from our previous synthesis in several important ways: (1) it targets the 19*R*-epimer necessary for application of a Mitsunobu macrocyclization reaction, as reported by Paterson;^{6h} (2) the starting material (**5** or L-ascorbic acid) contains the stereochemical information necessary for both the C19 and C20 chirality centers; and (3) the epoxide is incorporated early in order to direct the asymmetric allylation reaction to be used for coupling the two major fragments of the molecule.

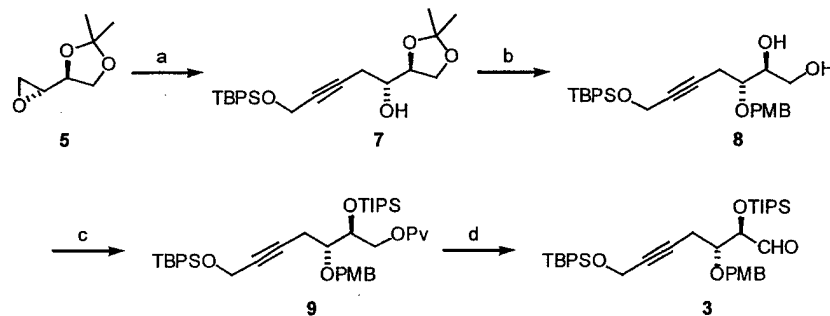
The synthesis of fragment **3** is outlined in Scheme 2. Opening of the epoxide with the anion generated from TBPS propargyl ether gave **7**, which was converted into aldehyde **3** via a series of protection and deprotection steps. Protection of the secondary alcohol as its PMB ether is followed by acetonide deprotection¹¹ to give diol **8**. Selective pivaloylation of the primary alcohol, silylation of the secondary alcohol, pivaloate ester reduction, and Swern oxidation gave compound **3**.

The synthesis of sulfone **4** (Scheme 3) started with diene **6**, available in three steps from (*R*)-glycidol, as previously described.^{6k} Ring closing olefin metathesis and deprotection gives alcohol **10**, which can be coupled with 1-phenyl-1*H*-tetrazole-5-thiol under Mitsunobu conditions to give sulfide **11**. The oxidation of the sulfide to the desired sulfone, however, proved troublesome. A survey of the usual oxidizing agents, showed ammonium molybdate/H₂O₂¹² to give the best results.

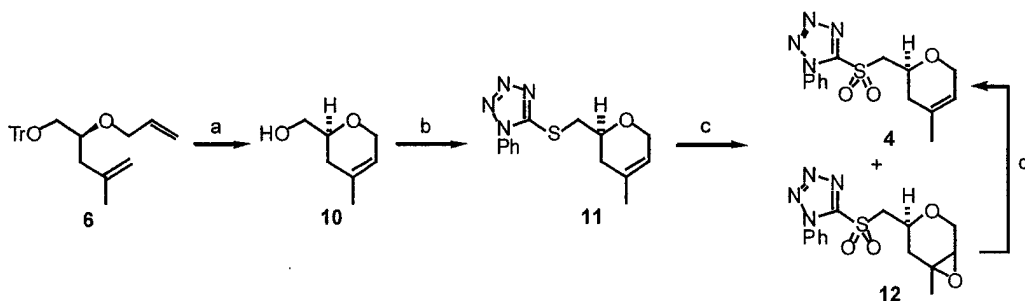
Still, although the initial oxidation of the sulfide to a sulfoxide was extremely facile, further oxidation to give sulfone **4** proved quite difficult. In fact, over oxidation of the alkene in **4** to the epoxide (**12**) was competitive with sulfoxide oxidation, typically yielding a mixture of sulfoxide, sulfone (**4**), and epoxysulfone (**13**) products. Fortunately, treatment of **12** with PPh₃/I₂ cleanly converted the epoxide back to the desired alkene.¹³ Thus, the ammonium molybdate catalyzed oxidation was allowed to run until the sulfoxide was completely consumption, giving a 1.1:1 ratio of **4**:**12**, the later of which could be recycled back to sulfone **4**, ultimately giving an 80% yield of sulfone, from **11**.

The coupling of fragments **3** and **4** (Scheme 4) was accomplished using the Kocienski-modified Julia coupling procedure.⁷ Our initial attempt involved deprotonation of sulfone **4** with KHMDS in DME followed by addition of aldehyde **3**, giving the crude product in a respectable 80% yield, but with a disappointing 1:1.3 *trans*:*cis* isomeric ratio. Fortunately, substituting DMF for DME provided the product in an equivalent 81% yield, but with an improved 5:1 *trans*:*cis* ratio. Selective removal of the TBPS group with NaOH in refluxing MeOH¹⁴ yielded a propargylic alcohol that was reduced with Red-Al¹⁴ to give allylic alcohol **14**. A Sharpless asymmetric epoxidation reaction¹⁵ provided the epoxy-alcohol, which could be oxidized to give the C15–C28 fragment of laulimalide (**2**).¹⁶

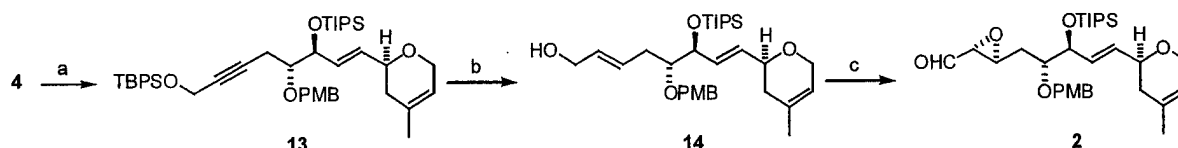
In summary, we have reported an improved synthesis of the C₁₅–C₂₈ fragment of the microtubule-stabilizing agent laulimalide. Salient features include: (1) the use



Scheme 2. (a) TBPSOCH₂C≡CH/*n*-BuLi, THF, BF₃·OEt₂, –78°C (82%); (b) (i) PMB–Br, KHMDS, DMF, 0°C, (ii) 1,3-propanedithiol (6 equiv.), BF₃·OEt₂, –78°C (71% over two steps); (c) (i) (CH₃)₃COCl, Et₃N, CH₂Cl₂ (93%), (ii) TIPS–OTf, 2,6-lutidine, DMF (95%); (d) (i) DIBALH, CH₂Cl₂, –78°C (98%), (ii) (COCl)₂, DMSO, –78°C, then Et₃N, –78°C to rt (88%).



Scheme 3. (a) (i) Grubbs' catalyst, CH₂Cl₂, (95%), (ii) TFA, MeOH (81%); (b) DIAD, PPh₃, 1-phenyl-1*H*-tetrazole-5-thiol (85%); (c) (NH₄)₆Mo₇O₂₄, H₂O₂, EtOH (**4**, 45%; **13**, 40%); (d) PPh₃, I₂, CH₃CN (87%).



Scheme 4. (a) KHMDS, DMF, then **3** (81%; 5:1, *trans:cis*); (b) (i) 10% NaOH in MeOH, reflux (87%), (ii) Red-Al, ether (85%); (c) (i) (+)-DIPT, TBHP (84%; 85% d.e.), (ii) (COCl)₂, DMSO, –78°C, Et₃N, –78°C to rt (85%).

RCM chemistry for the preparation of the terminal dihydropyran ring; (2) the incorporation of both the C19 and C20 stereochemistries in the starting material; and (3) the application of a Julia/Kocienski coupling for the successful addition of the dihydropyran side chain. Further work toward the synthesis of laulimalide is underway.

Acknowledgements

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References

- (a) Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644–3646; (b) Quinoa, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642–3644; (c) Jefford, C. W.; Bernardinelli, G.; Tanaka, J.-i.; Higa, T. *Tetrahedron Lett.* **1996**, *37*, 159–162.
- Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653–660.
- (a) ter Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243–250; (b) Kowalski, R. J.; Giannakakou, P.; Gunasekera, S.; Longley, R. E.; Day, B. W.; Hamel, E. *Mol. Pharmacol.* **1997**, *52*, 613.
- (a) Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744–8745; (b) Long, B. H.; Carboni, J. M.; Wasserman, A. J.; Cornell, L. A.; Casazza, A. M.; Jensen, P. R.; Lindel, T.; Fenical, W.; Fairchild, C. R. *Cancer Res.* **1998**, *58*, 1111–1115.
- Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534–2541.
- (a) Ghosh, A. K.; Mathivanan, P.; Cappiello, J. *Tetrahedron Lett.* **1997**, *38*, 2427–2430; (b) Shimizu, A.; Nishiyama, S. *Tetrahedron Lett.* **1997**, *38*, 6011–6014; (c) Shimizu, A.; Nishiyama, S. *Synlett* **1999**, 1209–1210; (d) Mulzer, J.; Hanbauer, M. *Tetrahedron Lett.* **2000**, *41*, 33–36; (e) Ghosh, A. K.; Wang, Y. *Tetrahedron Lett.* **2000**, *41*, 2319–2322; (f) Ghosh, A. K.; Wang, Y. *Tetrahedron Lett.* **2000**, *41*, 4705–4708; (g) Dorling, E. K.; Ohler, E.; Mulzer, J. *Tetrahedron Lett.* **2000**, *41*, 6323–6326; (h) Ghosh, A. K.; Wang, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6323–6326; (i) Paterson, I.; De Savi, C.; Tudge, M. *Org. Lett.* **2001**, *3*, 213–216; (j) Nadolski, G. T.; Davidson, B. S. *Tetrahedron Lett.* **2001**, *42*, 797–800; (k) Messenger, B. T.; Davidson, B. S. *Tetrahedron Lett.* **2001**, *42*, 801–803; (l) Ghosh, A. K.; Wang, Y. *Tetrahedron Lett.* **2001**, *42*, 3399–3401; (m) Dorling, E. K.; Ohler, E.; Mantoulidis, A.; Mulzer, J. *Synlett* **2001**, 1105–1108; (n) Lee, H. W.; Jeong, C. S.; Yoon, S. H.; Lee, I. Y. C. *Bull. Kor. Chem. Soc.* **2001**, *22*, 791–792; (o) Mulzer, J.; Ohler, E. *Angew. Chem., Int. Ed.* **2001**, *40*, 3842–3846; (p) Paterson, I.; De Savi, C.; Tudge, M. *Org. Lett.* **2001**, *3*, 3149–3152.
- (a) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 26–28; (b) Kocienski, P. J.; Bell, A.; Blakemore, P. R. *Synlett* **2000**, 365–366.
- Le Merrer, Y.; Gravier-Pelletier, C.; Dumas, J.; Depezay, J. C. *Tetrahedron Lett.* **1990**, *31*, 1003–1006.
- Although based upon the methods reported in Ref. 8, the following modified procedure was used:
- Recent reviews include: (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413–4450; (b) Furstner, A. *Top. Catal.* **1997**, *4*, 285–299; (c) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2036–2055.
- Konosu, T.; Oida, S. *Chem. Pharm. Bull.* **1991**, *39*, 2212–2215.
- Blakemore, P. R.; Kocienski, P. J.; Marzcek, S.; Wicha, J. *Synthesis* **1999**, 1209–1215.
- Garlaschelli, L.; Vidari, G. *Gazz. Chim. It.* **1987**, *117*, 251–253.
- Hatakeyama, S.; Irie, H.; Shintani, T.; Noguchi, Y.; Yamada, H.; Nishizawa, M. *Tetrahedron* **1994**, *50*, 13369–13376.
- Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765–5789.

16. Selected data for compound 2: ^1H NMR δ 8.87 (1H, d, $J=6.3$ Hz), 7.24 (2H, d, $J=8.6$ Hz), 6.84 (2H, d, $J=8.6$ Hz), 5.73 (2H, m), 5.41 (1H, brs), 4.71 (1H, d, $J=11.4$ Hz), 4.43 (1H, d, $J=11.4$ Hz), 4.38 (1H, m), 4.16 (2H, m), 4.02 (1H, dt, $J=9.9, 3.3$ Hz), 3.54 (1H, dt, $J=8.4, 3.3$), 3.26 (1H, ddd, $J=6.2, 5.2, 1.9$ Hz), 3.06 (1H, dd, $J=6.2, 1.9$ Hz), 2.07 (1H, ddd, $J=14.6, 8.7, 5.1$ Hz), 2.03 (1H, m), 1.89 (1H, brd, 16.7 Hz), 1.70 (3H, s), 1.66 (1H, ddd, $J=14.6, 6.3, 3.8$ Hz); ^{13}C NMR δ 198.1, 159.3, 133.0, 131.2, 130.6, 130.3, 129.6, 119.79, 113.8, 80.2, 75.6, 73.3, 72.3, 65.5, 59.0, 55.0, 55.0, 35.5, 32.4, 22.9, 18.1, 12.4; HRFABMS (MNBA+NaI) calcd for $\text{C}_{31}\text{H}_{48}\text{NaO}_6\text{Si}$: 567.3118; found: 567.3105.